Genotypic and Phenotypic Components Of Alpine Plant Response to Acid Rain

By Mary A. McKenna

GENOTYPIC AND PHENOTYPIC COMPONENTS OF ALPINE PLANT RESPONSE TO ACID RAIN

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DR. MARY A. MCKENNA
BIOLOGY DEPARTMENT
HOWARD UNIVERSITY
WASHINGTON, D.C. 20059

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INTRODUCTION

Global atmospheric change as a result of anthropogenic processes has accelerated in recent years, and debate over the effects of specific atmospheric changes on the earth's biota has increased. The phenomenon of "acid rain" has provoked considerable research and public debate. Acid rain is defined as rainfall with pH values below 5.6, usually resulting from atmospheric accumulation of oxides of sulfur and nitrogen. The effects of acid rain have been intensively studied in many terrestrial and aquatic communities. These studies have demonstrated a range of effects that vary depending on the organism, its developmental stage and other ecological factors (Defries and Malone, 1989; Linthurst, 1984).

This study is part of an ongoing effort to explore the effects of acid rain in populations of alpine and sub-alpine plants in the central Rocky Mountains. The research site is the Glacier Lakes Ecosystem Experiments Site (GLEES), in the Snowy Mountain Range, approximately 30 miles from Laramie, Wyoming. Previous studies of more than 20 herbaceous species at GLEES suggest that relatively quick and efficient estimates of plant sensitivity to low pH can be made by laboratory assays of pollen sensitivity (McKenna, 1990). These studies have also demonstrated reduced pollen germination and growth in response to low pH in a variety of species. Field pollination studies at the GLEES site have shown that some species (*Aquilegia caerulea*) produce fewer seeds and lighter seeds after treatment with simulated acid rain (McKenna, 1990, 1992). Other species may be more tolerant, however; studies with *Erythronium grandiflorum* indicate less sensitivity to low pH during pollen germination and little effect on the seed set of plants exposed to acid rain (McKenna, 1990, 1992). In 1990 a long-term study was initiated to investigate the effects of acid rain on vegetative and reproductive processes in *Aquilegia caerulea*. *Aquilegia* plants exposed to acid rain simulant show consistent reductions in vegetative growth parameters compared to plants exposed to ambient rain simulant (McKenna, 1990, 1992). One major objective of the present study was to further study the long term growth and reproduction of *Aquilegia* plants under simulated acid rain stress.

A second objective of the present study was to initiate a new long-term experiment to study the effects of simulated acid rain on growth and reproduction in *Penstemon whippleanus*. Laboratory studies of *Penstemon* pollen germination and growth were carried out in 1991, using pollen from three elevational sites. A significant decrease in germination was observed below pH 5.5, and germination dropped to 4 % at ph 3.5 (McKenna, 1992). Average pollen tube length also decreased in medias with pH below 5.5. Pollen from all three

populations demonstrated a similar response to pH levels, although the pollen from the lowest elevation site (2500 m) had lower germination rates than pollen from the other sites (McKenna, 1992).

Hand pollination studies were also carried out in 1991 to investigate the effect of simulated acid rain on fruit development and seed set in a natural population of *Penstemon whippleanus* (mid-elevational site, 3000 m). Flowers were hand pollinated 24 hours after spraying with either an acid rain simulant (pH 3.5) or an ambient rain simulant (pH 5.6). Although we were able to harvest more than 95 % of the fruits developing from our pollinated flowers, only 10 % of the fruits harvested had seeds. We observed twice as many seeds in fruits derived from the ambient rain simulant (McKenna, 1992). The present study was designed to provide more natural simulation of acid and ambient precipitation by spraying whole plants rather than aiming the spray directly at the sexual parts of the flower. In addition, we designed the present study with a minimum of floral manipulation to avoid possible disruption of fertilization due to the physiological stresses of destamination and corolla removal and the microenvironmental changes resulting from the use of pollinator exclusion bags.

MATERIALS AND METHODS

Effects of simulated acid rain on *Aquilegia caerulea*

This experiment was designed to expand and extend the research data collected at the Meadow Creek population site in 1989, 1990 and 1991. Individual plants marked in 1991 were identified at emergence and relabelled for inclusion in one of three experimental treatment groups : spray with acid-rain simulant, spray with ambient-rain simulant, and a non-spray treatment. Initial measurements were made of crown height and width, and the five stems were randomly selected and labelled for repeat measurements of stem height. A pressurized hand-held sprayer was used to spray all plants with 350 ml of rain simulant twice a week for 4 weeks. Growth measurements were taken weekly. Flower and fruit development was monitored weekly and pollen samples were collected when available for pollen growth analysis.

Effects of simulated acid rain on *Penstemon whippleanus*

A new study was initiated in 1992 to investigate the effect of simulated acid rain on growth and reproduction in a natural population of *Penstemon whippleanus*. This species was chosen for a intensive study because it has a relatively closed floral morphology, with sexual parts protected within the corolla. Our previous species on acid rain effects on growth and reproduction have involved flowers with exposed sexual parts (*Erythronium* and *Aquilegia*). We are interested in the possible influence of floral morphology on reproductive response to acid rain. *Penstemon whippleanus* is a rhizomatous perennial that sends up several single-stalked ramets per genet. Vegetative growth can be quantified by measuring the size of each ramet, and flower and fruit production can be easily monitored. Additional advantages to this species are that the size of individual ramets allows for each stem to be sprayed individually, and floral size is appropriate for hand pollination studies.

A long-term experimental population of *Penstemon whippleanus* was established at a site approximately 20 meters above Brooklyn Lake. Three treatment groups were established in the population: acid rain simulant spray, ambient rain simulant spray and non-spray controls. Sixty three individual stems (ramets) were labelled for inclusion in each treatment group, and the position of all ramets was mapped and recorded. We tried to avoid combining different treatments within one genet, and we tried to assign only one ramet per genet to each treatment. Determination of genet size was made on the basis of above ground location of ramets. We wished to avoid confounding our experimental treatment effects by disturbing the plants through excavation to establish rhizome connections.

Formulation of rain simulant solutions was developed to approximate natural background ion concentrations measured in precipitation at GLEES (McKenna, 1990). For rain-simulant treatments, each stem was sprayed with 160 ml of acid-rain simulant (pH 3.5) or ambient rain simulant (pH 5.6) three times weekly, using a pressurized hand-held sprayer. Measurements of stem height, and the number of flower buds, open flowers and fruits were taken over four weekly intervals. At the conclusion of the experiment, each stem was individually severed from the rhizome, placed in a labelled paper bag and dried in a laboratory oven for 48 hours at 60 degrees Centigrade. Measurements were made of dry weight of plants and fruits.

Two weeks after the spray treatments were initiated, hand pollinations were carried out on thirty three plants in each treatment using pollen donors collected

from an adjacent population. Stigmas were coated with abundant pollen from mature anthers held in forceps and rubbed across the stigmatic surface. Flowers were not destaminated or bagged in order to avoid physiological disturbances and microhabitat modifications that may result in poor fertilization and fruit set in this species (McKenna, 1992). Pistils of all pollinated flowers were collected two days after pollination and preserved in 70% ethanol for microscopic examination of pollen tube growth and ovule development. An additional 30 pistils were collected and preserved from thirty plants in each treatment, for examination of pollen load, tube growth and ovule development of flowers exposed to natural pollinators during the period of our hand pollination study. A second group of hand pollinations was initiated one week later. A total of 20-30 flowers were pollinated in each treatment using the same methods described above. The pistils from these pollinations were allowed to develop naturally on the plant; fruits from these pollinations were collected two weeks later when all experimental stems were harvested.

Pollen samples from all experimental treatments were collected three weeks after the initiation of the spray regime and transported to the laboratory for analysis of germination and growth *in vitro*. Anthers from all treatments were collected just prior to dehiscence, and placed in a closed petri dish under fluorescent lights until the pollen was released. Pollen was germinated and grown in Brewbaker/Kwack media (Brewbaker and Kwack, 1963) adjusted to pH 3.5 or pH 5.5 using techniques described in earlier studies (McKenna, 1990, 1992).

RESULTS

Aquilegia caerulea plants exposed to the acid rain simulant demonstrated a marked reduction in vegetative growth. The mean height of marked stems from plants in the acid spray treatment was significantly lower than stem heights of plants from the ambient or non-spray treatment on all measurement dates (Table One). After four weeks of spray treatments, the stem height of plants receiving the ambient rain simulant was significantly greater than plants in the non-spray treatment (Table One). After four weeks of spray treatments, the mean stem height of plants in the ambient-spray treatment is greater than non-spray plants and acid-spray plants (Table One). Plants in the acid-spray treatment also showed reduced plant height and crown diameter than plants in the other treatments (Table Two and Table Three).

The plant height and stem height of plants in the acid-spray treatment were also reduced in comparison to previous years. Mean stem height of acid-spray

plants in the second week of August 1990 was 13.77 cm; mean stem height of the same plants in the second week of August 1992 was 9.67 cm. Plants in the ambient-spray treatment did not show a comparable reduction in growth from previous measurements. Mean stem height of ambient-spray plants in the second week of August 1990 was 16.26 cm; mean stem height of the same plants in the second week of August 1992 was 15.42 cm.

A reduction in reproductive effort was also observed in *Aquilegia* plants exposed to acid rain simulant (Table Four). By the second and third week of the experiment, plants exposed to the acid rain simulant produced only half as many buds as plants exposed to the ambient rain simulant. By the fourth week of the experiment, plants exposed to the acid rain simulant showed a 72 percent reduction in bud production and a 58 percent reduction in flower production compared to plants exposed to the ambient rain simulant. The reproductive effort demonstrated by *Aquilegia* plants in the non-spray treatment was at levels intermediate between plants in the acid-spray and ambient-spray treatments. Reduced levels of total bud/flower production were seen in acid spray plants (413) compared to the total bud/flower production of ambient spray plants (817) and non-spray plants (672).

Pollen collected from *Aquilegia* plants in all treatments showed high viability in medias at pH 3.5 and pH 5.5 (non-spray: 95.3% ; ambient-spray: 89% ; acid-spray: 85%). Very low germination rates were observed with *Aquilegia* pollen from all treatments in medias at pH 3.5 and 5.5 (non-spray: 4% ; ambient-spray: 4% ; acid-spray: 2%). The low germination rates are most likely a result of immature pollen grains since the anthers were collected for this experiment before they were fully developed. Although the anthers continued to ripen in laboratory before the pollen was removed, we suspected that the pollen might be immature since it did not release easily from the anthers into the experimental wells.

Plant height in *Penstemon whippleanus* showed little change as the season progressed, and there was no observable effect of spray treatments on the mean height of individual stems (Table Five). No significant differences were seen in the dry weight of plants harvested from each of the treatments (Table Seven). No differences were seen in the volume or pattern of bud, flower and fruit production among plants in the three experimental treatments (Table Six) throughout the duration of the study. Total bud/flower production was equal in each treatment group (acid-spray : 63; ambient-spray: 65; non-spray: 66). There was no sigificant difference in the number of fruits harvested from plants in each experimental treatment, and the mean weight of fruits derived from the three treatments did not differ significantly (Table Seven).

Hand pollinations of flowers in each treatment resulted in a low fruit set (acid-spray: 24%; ambient-spray: 21%; non-spray: 19%). No significant differences were seen in the mean number of seeds per fruit (acid: 25.5; ambient 27.2) or the mean weight of seeds (acid: 58.8 ug; ambient: 61.3 ug) in fruits derived from hand pollination of flowers in the ambient-spray and acid-spray treatments. Fruits derived from hand pollination of flowers in the nonspray treatment had greater average seed number (35.0) and mean seed weight (108.6 ug) than fruits from the other two treatments.

Pollen collected from *Penstemon whippleanus* plants in experimental treatments demonstrated high viability in medias at pH 5.5 and pH 3.5 (Table Eight, Nine and Ten). Pollen from flowers in the three experimental treatments demonstrated a similar response to media pH levels. Germination percentages of pollen in media at pH 3.5 (0.7 - 2%) were significantly lower than the germination percentages of pollen in media at pH 5.5 (39% - 49%).

DISCUSSION

Vegetative growth processes in *Aquilegia caerulea* appear to be significantly affected by treatment with acid rain simulant. These effects may be cumulative, since plant heights and stem heights measured in the acid-spray plants in 1992 are reduced in comparison to the 1990 values on all measurement dates. Year to year variation in general environmental factors influencing the timing and vigor of plant growth may account for some of this growth reduction, since the height of ambient-spray plants in 1992 was also reduced (by 5%) compared to the values in 1990. Comparative growth reductions were much greater (15-30%) among in the acid-spray plants however. Potential causes of the growth reductions seen in plants treated with acid rain simulant include direct physical or chemical injury of tissues, disruption of biochemical processes including photosynthesis and respiration, and increased susceptibility to pathogens (Treshow, 1984; Hutchinson and Meema, 1987). The ambient rain spray may have stimulated vegetative growth, since we observed greater stem height and plant height of plants in this treatment than either the non-spray plants or the acid-spray plants. Increased growth of ambient-spray plants in comparison to non-spray plants may result from increased access to water or possible enrichment effects of the background ions used to formulate the rain simulant solutions.

The marked reduction in the reproductive effort of *Aquilegia* plants in the acid-spray treatment suggests that atmospheric processes may have long term ecological and evolutionary consequences for this species. The treatment effects

on reproductive effort may also be cumulative; in 1990 the acid-spray plants produced one-third fewer flowers than the ambient-spray plants, while in 1992 the acid-spray plants produced only half as many flowers as the ambient-spray plants. Increased flower production in the ambient-spray treatment between 1990 and 1992 accounts for some of this increase in differential flower production between the treatments. The flower production in acid-spray plants decreased by 9 percent between 1990 and 1992, while flower production in ambient spray plants increased by 22 percent.

Reductions in vegetative growth of plants are frequently accompanied by reductions in flower production primarily due to energy limitations, but some species respond to environmental stress by increasing reproductive effort (Grime, 1982; Levitt, 1989). Previous studies in this population of *Aquilegia caerulea* have demonstrated reduced seed production and reduced seed weight in plants exposed to acid rain simulant prior to hand pollination (McKenna, 1992). The combined effects of decreased flower production and decreased seed production in individuals exposed to the acid rain simulant suggests that acid rain stress may seriously impact the reproductive potential of *Aquilegia caerulea.*

The spray treatments appear to have little effect on pollen viability as measured by the Alexander's stain (Alexander, 1969). Similar pollen viability values in medias at pH 3.5 and pH 5.5 were obtained from *Aquilegia* plants in previous treatment years (ambient-spray: 89%; acid-spray: 82%). The use of immature pollen grains led to low pollen germination rates from all treatment groups in this study. In earlier studies of plants from the same population (McKenna, 1989, 1992), we have demonstrated significant reductions in germination rates of *Aquilegia* pollen in media at pH 3.5. Significant reductions in germination at pH 3.5 are seen in pollen from both acid-spray and ambient-spray treatments (McKenna, 1992).

In *Penstemon whippleanus* our data showed no effect of spray treatments on the height or final dry weight of individual stems (ramets) within the experimental population. The height of individual stems was remarkably consistent between the three experimental groups and throughout the duration of the four-week experiment. Stem heights changed by only 2.0-2.6 cm in each treatment group during the four week interval. Stem height in this species may be predetermined by energy stored in the rhizome during the previous growing season. We began spraying and measuring the stems after they had achieved more than 90 percent of their maximum height. It is unlikely that the spray treatments would have a significant effect on stem height or weight if the treatments began after the biochemical processes of plant growth were essentially completed for the season.

There were no apparent effects of the spray treatments on bud, flower or fruit production in *Penstemon whippleanus* throughout the duration of the study. Our study interval appeared to span the period of reproductive activity for this species, since bud production declined to very low levels by the last measurement date. Production of flowers and fruits was also remarkably consistent between the three experimental groups. The energy expended on reproductive effort may be closely related to stem height, and this life history characteristic may also be largely predetermined by energy stored in the rhizome during the previous growing seasons.

Pollen viability in *Penstemon whippleanus* appears to be unaffected by spray treatments; similar high pollen viability values in medias at pH 3.5 and pH 5.5 were obtained from *Penstemon* plants from other populations in previous years (McKenna, 1989, 1992). The spray treatments appear to have no effect on the pollen response to media pH since pollen from both spray treatments showed a nearly identical germination rate in media at pH 5.5, and a similar reduction in germination rate in media at pH 3.5. In our 1991 study of pollen from *Penstemon* populations at three elevational sites, the pollen germination values in media at pH 3.5 (2.7 - 5.2 %) were marginally higher than the values obtained in the present study.

The low fruit set observed in this study following hand pollinations of *Penstemon whippleanus* flowers in the three spray treatments is consistent with the results of a similar study in 1991 carried out in a different population of *Penstemon whippleanus*. Fruit set in the 1991 study of plants from the Mountain Meadow population was only 9 percent in flowers sprayed with acid-rain simulant and 10% in flowers sprayed with ambient-rain simulant. The doubling of fruit set percentages seen in the present study may reflect the institution of experimental design changes to reduce unnecessary sources of physiological stress during fertilization and fruit development. These design changes include eliminating the need to destaminate and bag flowers and eliminating the direct spraying of pistils with rain simulant solutions.

The observed low percent fruit set of hand-pollinated *Penstemon whippleanus* flowers in the non-spray treatment suggests that factors other than the spray treatments themselves are responsible for the low proportion of fruits with mature seeds. These may include general factors such as sexual incompatibility systems or energetic constraints on fruit and seed development. It is also possible that some aspect of the hand pollination process is responsible for the low fruit set such as physiological effects due to large pollen loads, direct damage to the style or stigma during the pollination process or attempts to pollinate flowers before or after the period of maximal stigmatic/stylar receptivity. In 1992, we collected

and preserved pistils of flowers in each treatment group after exposure to natural sources of pollination and after hand pollination. Through the use of uv-fluorescence microscopy, we are currently examining the stigma and style of these specimens to evaluate pollen loads and pollen growth following natural pollination and hand pollination in this species.

Further studies with *Penstemon whippleanus* will be required before we can draw any conclusions regarding its possible tolerance to acid rain during vegetative growth and reproduction. Treatment effects from the 1992 study may be observed in subsequent years if growth and reproduction are a function of energy stored in previous years. Future spray treatments will be initiated as soon as ramets emerge from the vegetative buds. We are currently engaged in mapping the rhizome connections linking all ramets within each genet in this population, so that spray treatments can be applied uniformly to all ramets within each genet. This design will prevent the possibility that translocation between ramets could alleviate or exacerbate the environmental stresses applied to individual ramets (Salzman and Parker, 1988).

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TABLE ONE

Aquilegia caerulea
1992

	Mean Stem Height[1]		
Date	Acid (n=75)	Ambient (n=75)	Non-Spray (n=75)
7-23	9.4[a] (3.0)	11.5[b] (4.2)	11.2[b] (3.6)
7-30	9.4[a] (3.0)	11.6[b] (4.4)	11.6[b] (4.0)
8-11	9.7[a] (2.9)	15.4[c] (6.2)	11.5[b] (3.8)

[1] Means in centimeters (plus standard deviation)

NOTE: Means with different letters are significantly different at $p \leq .05$

TABLE TWO

Aquilegia caerulea
1992

Date	Mean Plant Height[1] Acid (n=15)	Ambient (n=15)	Non-Spray (n=15)
7-15	12.4[2] (3.1)	16.3 (5.8)	14.9 (4.8)
7-23	14.8 (3.7)	17.0 (6.6)	16.0 (5.0)
7-30	13.7 (4.4)	18.2 (8.0)	16.9 (5.5)
8-11	15.5 (5.7)	18.2 (7.3)	16.5 (5.7)

[1] Means in centimeters (plus standard deviation)

[2] n=12

TABLE THREE

Aquilegia caerulea
1992

	Mean Crown Diameter[1]		
Date	Acid (n=15)	Ambient (n=15)	Non-Spray (n=15)
7-15	22.8 (8.3)	25.2 (11.2)	23.1 (8.6)
7-23	26.8 (11.3)	28.8 (14.0)	25.8 (10.2)
7-30	27.5 (10.5)	28.1 (14.6)	26.6 (11.6)
8-11	25.3 (10.5)	31.3 (15.2)	27.1 (11.6)

[1] Means in centimeters (plus standard deviation)

TABLE FOUR

Aquilegia caerulea
1992

	TOTAL NUMBER OF BUDS, FLOWERS AND FRUITS								
	Acid			Ambient			Non-Spray		
Date	Buds	Flowers	Fruits	Buds	Flowers	Fruits	Buds	Flowers	Fruits
7-15	58	1	0	77	2	0	66	0	0
7-23	115	2	0	238	2	0	167	4	0
7-30	113	24	0	207	31	0	195	21	0
8-11	18	82	0	64	196	0	76	143	0

TABLE FIVE

Penstemon whippleanus
1992

Date	Mean Plant Height[1] Acid (n=52)	Ambient (n=53)	Non-Spray (n=57)
7-10	22.9 (5.5)	21.8 (4.8)	21.7 (5.3)
7-17	24.1 (5.8)	23.6 (5.2)	23.1 (5.5)
7-23	25.3 (5.8)	23.9 (5.2)	24.2 (5.7)
7-31	24.6 (5.6)	23.8 (5.2)	24.3 (5.6)
8-07	24.4 (6.0)	24.0 (5.3)	23.8 (5.4)

[1] Means in centimeters (plus standard deviation)

TABLE SIX

Penstemon whippleanus
1992

	AVERAGE NUMBER OF BUDS, FLOWERS AND FRUITS PER PLANT								
	Acid (n=52)			Ambient (n=53)			Non-Spray (n=57)		
Date	Buds	Flowers	Fruits	Buds	Flowers	Fruits	Buds	Flowers	Fruits
7-10	15	0	0	15	0	0	14	0	0
7-17	12	3	0	13	4	0	14	4	0
7-23	7	9	0	6	9	0	6	8	0
7-31	2	8	6	1	9	5	3	7	5
8-07	0	7	6	2	6	7	3	7	8

TABLE SEVEN

Penstemon whippleanus
Final Harvest 8-10-92[1]

	Acid (n=50)	Ambient (n=52)	Non-Spray (n=57)
Plant weight (g)	0.46 (0.22)	0.44 (0.22)	0.42 (0.18)
Number of fruits/plant	6.4 (4.8)	5.2 (5.8)	5.4 (4.3)
Fruit weight (mg)	13.3 (7.2)	12.4 (7.7)	12.9 (11.9)

[1] Values represent mean (plus standard deviation)

TABLE EIGHT

Penstemon whippleanus
Pollen Germination 1992[1]

Treatment Group: Acid-spray Plants	Media pH 5.5	Media pH 3.5
Percent viable pollen	95.9 (3.5) n=15	98.2 (1.6) n=11
Percent pollen germination	39.0[a] (29.7) n=15	0.7[b] (1.0) n=15
Number of grains/well	396	308

[1] Values represent mean (plus standard deviation)

NOTE: Means with different letters are significantly different at $p \leq .05$

TABLE NINE

Penstemon whippleanus

Pollen Germination 1992[1]

Treatment Group: Ambient-spray Plants	Media pH 5.5	Media pH 3.5
Percent viable pollen	95.4 (2.1) n=15	98.7 (1.0) n=12
Percent pollen germination	39.7[a] (9.6) n=15	2.2[b] (5.8) n=15
Number of grains/well	407	339

[1] Values represent mean (plus standard deviation)

NOTE: Means with different letters are significantly different at $p \leq .05$

TABLE TEN

Penstemon whippleanus
Pollen Germination 1992[1]

Treatment Group: Non-spray Plants	Media pH 5.5	Media pH 3.5
Percent viable pollen	93.5 (8.5) n=15	97.2 (2.2) n=11
Percent pollen germination	49.0[a] (28.3) n=15	2.0[b] (6.1) n=15
Number of grains/well	340	401

[1] Values represent mean (plus standard deviation)
NOTE: Means with different letters are significantly different at $p \leq .05$